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Eutylenchus excretorius Ebsary & Eveleigh, 1981 (Nematoda: Tylodorinae) from Spain with approaches to molecular phylogeny of related genera

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Summary – Nematode surveys in indigenous vegetation in northern Spain revealed the presence of a nematode population of the genus *Eutylenchus* associated with moist sandy soils in the rhizosphere of common reed (*Phragmites* sp.) on the banks of the Tera river in Garray (Soria province). Morphological and morphometrical studies on this population fits with *Eutylenchus excretorius*, representing the first report for Spain and southern Europe and the fifth report in Europe after Germany, Poland, Czech Republic and Russia. SEM studies were carried out for the first time on this species and showed four lips separated by deep grooves. Each lip bears an elongated, flexible, recurved projection (seta) 12 (11-13) μm long, proximal third wide, gradually attenuating, distal end rounded. Molecular characterisation of *E. excretorius* using several genes is provided. The sequence of D2-D3 expansion segments of 28S rRNA gene of this population was identical to a previously studied sample from Germany. Phylogenetic analysis using D2-D3 of 28S rRNA and partial 18S rRNA gene sequences of tylenchid nematodes revealed that *E. excretorius* clustered with moderate support with *Cephalenchus hexalineatus.* The position of *E. excretorius* on majority consensus Bayesian phylogenetic tree reconstructed using heat shock protein 90 gene sequence was not well resolved.

Keywords – 18S rRNA, 28S rRNA, *Cephalenchus hexalineatus*, D2-D3, description, heat shock protein 90, morphology, morphometrics, new record, phylogeny, SEM, taxonomy.

During nematode surveys of indigenous vegetation in northern Spain, a nematode population of the genus *Eutylenchus* Cobb, 1913 was found for the first time in that country. The nematode was associated with moist sandy soils in the rhizosphere of common reed (*Phragmites* sp.) on the banks of the Tera river in Garray (Soria province), northern Spain. This population morphologically resembled *E. excretorius* Ebsary & Eveleigh, 1981, a fact that prompted us to undertake a detailed morphological and molecular comparative study with previous reported data. *Eutylenchus excretorius* was originally described from Canada and has subsequently been reported from several European countries.

Eutylenchus consists of a small group of migratory ectoparasites of aquatic vascular plants. The genus is characterised by the presence of four cephalic setae and includes six species: *E. africanus* Sher, Corbett & Colbran, 1966; *E. excretorius*; *E. fueguensis* Valenzuela & Raski, 1985; *E. gracilis* Gagarin, 2003; *E. setiferus* (Cobb, 1893) Cobb, 1913; and *E. vitiensis* Orton Williams, 1979. Nematodes of this rarely found and little known genus occur in moist sandy soils near streams and rivers in

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widely distributed areas of the world. Species of the genus have been reported on every continent with the exception of Antarctica, *viz*., in North and South America: Canada (Ebsary & Eveleigh, 1981) and Chile (Valenzuela & Raski, 1985); in Australia: Fiji Islands (Orton Williams, 1979), Solomon Islands (Ye & Geraert, 1997) and New South Wales (Sher *et al*., 1966); in Europe: Germany (Sievert & Sturhan, 1994), Poland (Brzeski, 1996), Czech Republic (Háněl, 2000) and Russia (Gagarin, 2003); in Asia: India (Husain & Khan, 1968), South Korea (Choi & Geraert, 1972; Choi *et al*., 1989), and Pakistan (Begum, 1996); and in Africa: Namibia (Van den Berg & Tiedt, 2006), Ivory Coast, Malawi, Nigeria and Zambia (Sher *et al*., 1966).

The taxonomic position of this genus is still controversial, since it has been included in different families or subfamilies by various authors (Andrássy, 1984; Maggenti *et al*., 1987; Siddiqi, 2000). Skarbilovich (1959) was the first to propose the family Atylenchidae Skarbilovich, 1959 and subfamily Atylenchinae Skarbilovich, 1959 for *Atylenchus* Cobb, 1913 and *Eutylenchus*. Sher *et al.* (1966) accepted this proposal and made a revision of the family. Paramonov (1970) suggested that *Atylenchus* and *Eutylenchus* belonged to the subfamily Atylenchinae in the family Tylenchidae Örley, 1880. Siddiqi (2000) placed *Eutylenchus* in a separate subfamily, the Eutylenchinae Siddiqi, 1986, in the family Atylenchidae. On the basis of lip region structure, arrangement of the uterus and spermatheca cells, Geraert and Raski (1987) grouped *Eutylenchus* together with *Cephalenchus* Goodey, 1962, *Tylodorus* Meagher, 1963 and *Campbellenchus* Wouts, 1977. In the classification proposed by Maggenti *et al.* (1987) *Eutylenchus* is placed, together with *Tylodorus*, *Macrotrophurus* Loof, 1958, *Cephalenchus* and *Campbellenchus*, in the subfamily Tylodorinae Paramonov, 1967 of the family Tylenchidae.

Evolutionary relationships of 82 species of tylenchids, including *E. excretorius* from Germany, were recently evaluated using the D2 and D3 expansion segments of 28S rRNA and different phylogenetic methods by Subbotin *et al.* (2006). However, the position of this species within Tylenchida was left uncertain and unresolved. In some trees, this species clustered, perhaps artificially, with the entomoparasitic nematode *Sphaerularia bombi* Dufour, 1837. Testing alternative hypotheses could not exclude a sister relationship with some representatives of Tylenchidae, but a potential sister relationship was rejected for *E. excretorius* and *Macrotrophurus*, another representative of the Tylodorinae *sensu* Maggenti *et al*. (1987). Thus, it was concluded that the phylogenetic position of *Eutylenchus* required further resolution through the study of additional genes and taxa.

Therefore, the objectives of this work were: *i*) to characterise morphologically and morphometrically the Spanish population of *E. excretorius* and compare with previous descriptions; *ii*) to characterise molecularly the Spanish population using the D2-D3 28S rRNA, partial 18S rRNA and heat shock protein 90 (hsp90) gene sequences; and *iii*) to reveal the phylogenetic position of *E. excretorius* within tylenchids using D2-D3 28S rRNA, partial 18S rRNA and hsp90 gene sequences. Several genes from some tylenchid species, including *Cephalenchus hexalineatus* (Geraert, 1962) Golden, 1971, *Psilenchus hilarulus* de Man, 1921 and *Psilenchus minor* Siddiqi, 1963, were also sequenced and included in the analysis.

Materials and methods

Nematode populations

Specimens of *E. excretorius* were obtained from moist sandy soil in the rhizosphere of common reed (*Phragmites* sp.) from the banks of the Tera river in Garray (Soria province), northern Spain (41°48′53.08″N latitude, 2°26′51.92″W longitude) at an altitude of 1011 m a.s.l.

Specimens of *C. hexalineatus* were recovered from soil samples shipped from: Florida, Goulds, plant host – Vriesea 'Splenreit' (CD 281); Florida, Homestead, plant host – *Guzmania rana*; Oregon, Dundee, host – *Malus* sp. (CD346). A population of *Helicotylenchus pseudorobustus* (Steiner, 1914) Golden, 1956 was extracted from soil samples collected at UC Riverside campus, and seed galls with *Anguina tritici* (Steinbuch, 1799) Filipjev, 1936 were kindly provided by Dr M. Madani.

Psilenchus hilarulus was obtained from clay-loam soil in the rhizosphere of grapevine in the Sierra de Bèrnia in Xalò (Alicante province), eastern Spain (38°39′47.25″N latitude, 0°02′55.76″W longitude) at an altitude of 896 m a.s.l. *Psilenchus minor* was obtained from moist sandy soil in the rhizosphere of unidentified graminaceous plants in the riverside of Guadalquivir river in Córdoba (Córdoba province), southern Spain (37°51′31.93″N latitude, 4°47′44.19″W longitude) at an altitude of 90 m a.s.l.

Nematodes were extracted from soil samples by magnesium sulphate centrifugal flotation (Coolen, 1979).

Light and scanning electron microscopy

Specimens for light microscopy (LM) were killed by gentle heat, fixed in a solution of 4% formaldehyde + 1% propionic acid, and processed to pure glycerin using Seinhorst's (1966) method. Specimens were examined using a Zeiss III compound microscope with Nomarski differential interference contrast at up to ×1000 magnification. Measurements were done using a camera lucida attached to a light microscope. Morphometric data were processed using Statistix 8.0 (NH Analytical Software, Roseville, MN, USA).

For scanning electron microscopy (SEM) studies, fixed specimens were dehydrated in a graded ethanol series, critical point dried, sputter-coated with gold and observed with a Jeol JSM-5800 microscope (Abolafia *et al*., 2002).

DNA extraction, PCR, cloning and sequencing

Nematode DNA from *E. excretorius* and *Psilenchus* spp. was extracted from single individuals as described by Castillo *et al*. (2003), whereas DNA from several specimens from the *C. hexalineatus* samples was extracted as described by Mundo-Ocampo *et al.* (2008). Amplification of rRNA genes and hsp90 from *E. excretorius* and *Psilenchus* spp. were performed as described by Castillo *et al*. (2003) and from *C. hexalineatus*, *H. pseudorobustus* and *A. tritici* samples as described by Tanha Maafi *et al.* (2003). Amplification of the hsp90 gene from *H. pseudorobustus* and *A. tritici* has been done from cDNA libraries of these species (Colbourne *et al*., 2007; Subbotin *et al.*, unpubl.), whereas amplification of this gene from *P. hilarulus*, *P. minor* and *E. excretorius* was done from genomic DNA. The following primers were used for amplification in the present study: D2-D3 of 28S rRNA: D2A (5′-ACAAGTACCGTGAGGGAAAGTTG-3′) and D3B (5′-TCGGAAGGAACCAGCTACTA-3′) (Subbotin *et al.*, 2006); partial 18S rRNA: G18SU (5′-GCT TGTCTCAAAGATTAAGCC-3′) and R18Tyl1 (5′-GG TCCAAGAATTTCACCTCTC-3′) (Chizhov *et al*., 2006); hsp90: U831 (5′-AAYAARACMAAGCCNTYT GGAC-3′) and L1110 (5′-TCRCARTTVTCCATGATR AAVAC-3′) (Skantar & Carta, 2005); ITS1-5.8S-ITS2: TW81 (5′-GTTTCCGTAGGTGAACCTGC-3′) and AB28 (5′-ATATGCTTAAGTTCAGCGGGT-3′) (Tanha Maafi *et al*., 2003).

PCR products were purified after amplification with Geneclean turbo (Q-BIOgene, Illkirch, France) or QIAquick (Qiagen, Valencia, CA, USA) gel extraction kits, quantified using a Nanodrop spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA) and used for direct sequencing (ITS, 18S, D2-D3 and hsp90 for *E. excretorius*, D2-D3 and hsp90 for *Psilenchus* spp.) or cloning (hsp90 for *H. pseudorobustus*, *A. tritici*, *C. hexalineatus* and D2-D3 and 18S for *C. hexalineatus*). The cloning protocol was as described by Tanha Maafi *et al.* (2003). Two clones were sequenced from each sample. The resulting products were purified and run on a DNA multicapillary sequencer (Model 3100 genetic analyser; Applied Biosystems, Foster City, CA, USA) at the University of Córdoba and University of California, Riverside, sequencing facilities. The newly obtained sequences were submitted to the GenBank database under accession numbers EU915486-EU915500 and as indicated on the phylogenetic trees.

Phylogenetic analyses

The newly obtained sequences for each gene were aligned using ClustalX 1.83 (Thompson *et al.*, 1997) with default parameters with corresponding published gene sequences, respectively (De Ley *et al*., 2005; Skantar & Carta, 2005; Holterman *et al.*, 2006; Subbotin *et al.*, 2006; Bert *et al*., 2008; Mundo-Ocampo *et al.*, 2008). Outgroup taxa for each dataset were chosen according to the results of previous published data (Skantar & Carta, 2005; Holterman *et al.*, 2006; Subbotin *et al.*, 2006; Bert *et al*., 2008). Sequence alignments of the protein coding gene were manually edited using GenDoc 2.5.0. (Nicholas *et al.*, 1997). Intron sequences were removed from the hsp90 gene alignment. Sequence datasets were analysed with Bayesian inference (BI) using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001). The best fit model of DNA evolution was obtained using the program MrModeltest 2.2 (Nylander, 2002) with the Akaike Information Criterion in conjunction with PAUP* 4b4a (Swofford, 2003). BI analysis under GTR + I + G model for each gene was initiated with a random starting tree and was run with four chains for 1.0×10^6 generations. Additional analysis for the protein coding gene was made with exclusion of most variable third nucleotide positions. The Markov chains were sampled at intervals of 100 generations. Two runs were performed for each analysis. The log-likelihood values of the sample points stabilised after approximately 1000 generations. After discarding burn-in samples and evaluating convergence, the remaining samples were retained for further analysis. The topologies were used to generate a 50% majority rule consensus

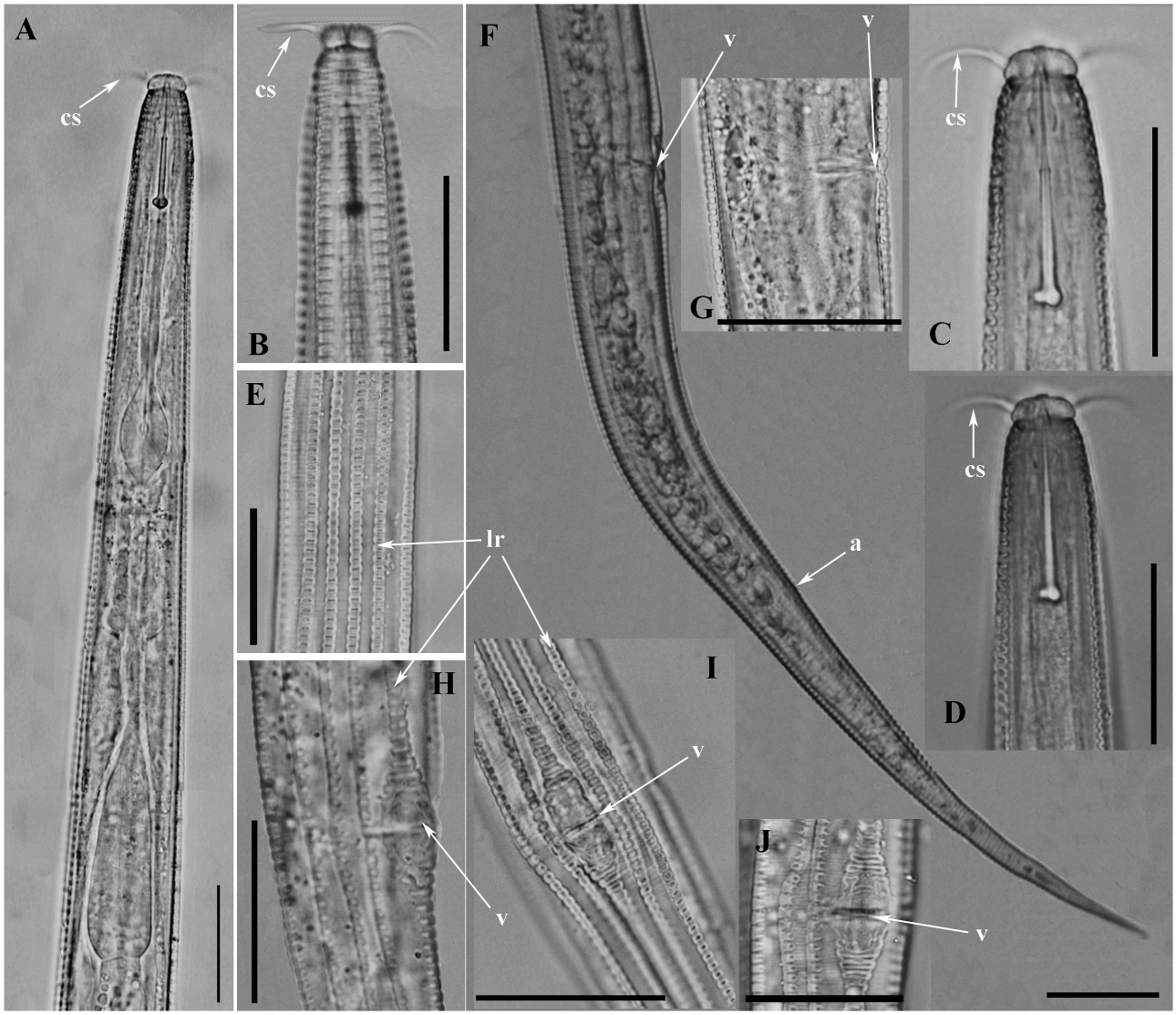

Fig. 1. *Light micrographs of female* Eutylenchus excretorius *Ebsary & Eveleigh, 1981. A: Anterior region (cs = cephalic setae); B-D: Lip region end showing cephalic setae (cs); E: Mid-body region showing transverse grooves and longitudinal ridges (lr); F: Posterior region showing vulva (v) and anus (a); G, H: Vulval region in lateral view showing vagina (v) and longitudinal ridges (lr); I, J: Vulval region in ventral view showing cuticular ridges forming advulval flaps (v = vulva). (Scale bars = 20 μm.)*

tree. Posterior probabilities (PP) are given on appropriate clades.

Eutylenchus excretorius Ebsary & Eveleigh, 1981
(Figs 1, 2)

Measurements

See Table 1.

Description

Female

Body elongate, tapering in neck region and gradually from vulva to a fine tail terminus. Habitus ventrally arcuate, usually in wide open C-shape when relaxed by gentle heat. Cuticle 1.0-1.5 μm thick; annuli 1.0-1.5 μm wide at mid-body formed by transverse grooves, bearing 12 equal, longitudinal ridges (2.5-3.0 μm wide). Lip re-

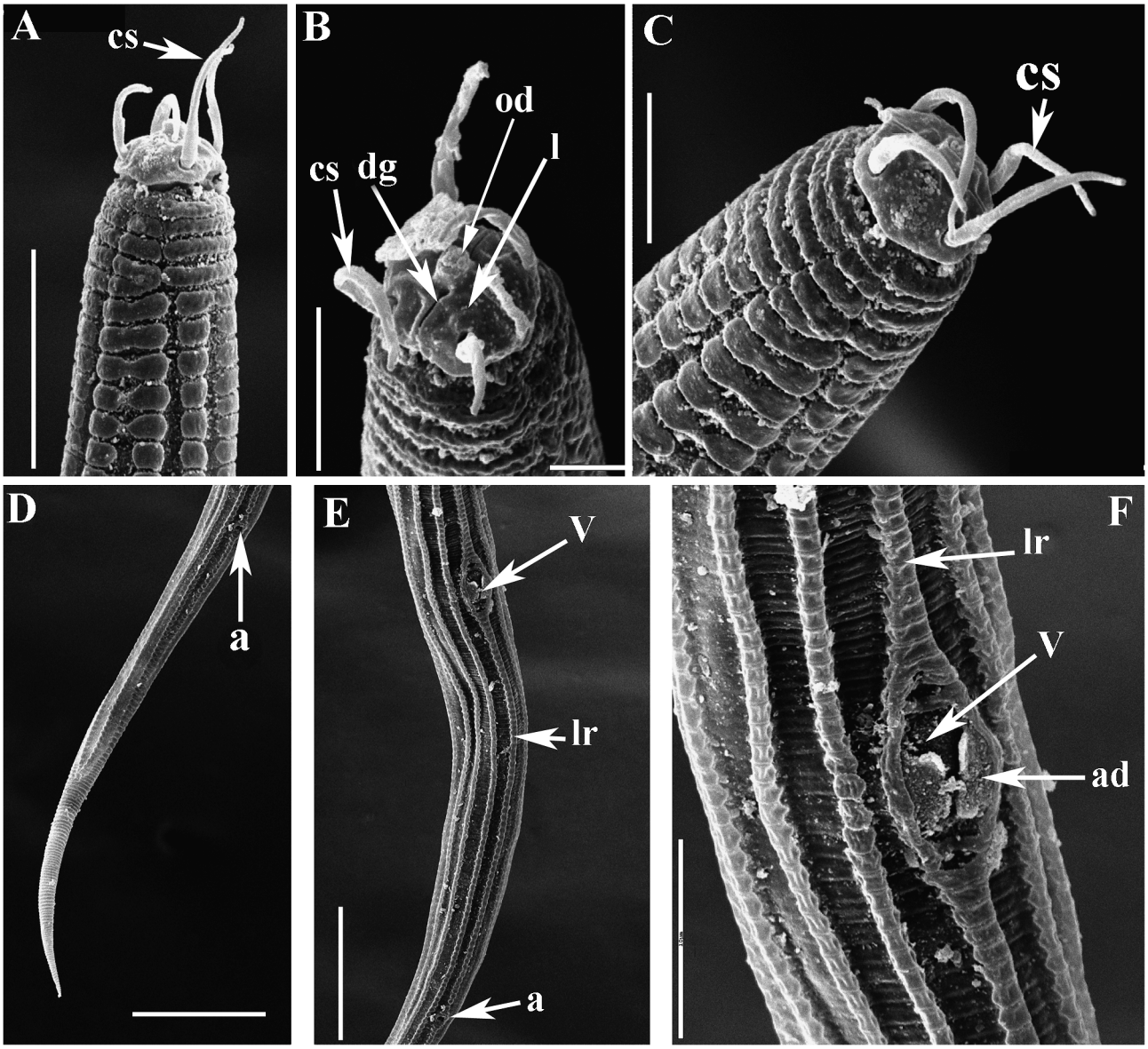

Fig. 2. SEM *micrographs of female* Eutylenchus excretorius *Ebsary & Eveleigh, 1981. A-C: Anterior ends in lateral and* en face *view showing oral disc (od), cephalic setae (cs) and deep grooves (dg) separating lips (l); D: Tail region showing anus (a); E: Vulval region (V), longitudinal ridges (lr) and anal region (a); F: Detail of vulva sowing advulval flaps (ad), vulva (V) and longitudinal ridges (lr). (Scale bars: A = 10 µm; B, C = 5 µm; D, E = 25 µm; F = 10 µm.)*

gion flattened 7.0 ± 0.4 (6.5-7.5) µm diam. × 2.5 ± 0.4 (2.0-3.0) µm high, clearly set off by constriction. SEM micrographs revealing presence of prominent, rounded, oral disc and four lips separated by deep grooves, the lateral grooves appearing as slits. Each lip bearing an elongated, flexible, recurved projection (seta) 8.9 ± 0.8 (8.0-10.0) µm long with proximal third wide then gradually attenuating to rounded distal end. Stylet moderately developed, conus thin, forming 44-45% of stylet length, knobs well developed, rounded, slightly backwardly directed. Dorsal pharyngeal gland orifice 2.0-2.5 µm from stylet base. Procorpus cylindrical, 27.0 ± 2.6 (23-30) µm long. Median pharyngeal bulb well developed, oval, 12.8 ± 1.3 (11-14) × 8.4 ± 0.5 (8-9) µm, valvular ap-

Table 1. *Morphometrics of female* Eutylenchus excretorius *Ebsary & Eveleigh, 1981 from a population found in a moist sandy soil in the rhizosphere of common reed (*Phragmites *sp.) on the banks of the Tera river, Garray (Soria province), northern Spain, and* Cephalenchus hexalineatus *(Geraert, 1962) Golden, 1971 from Florida and Oregon (USA). Measurements are in* μm *and in the form: mean* ± *standard deviation (range) coefficient of variation.*

Parameter	*Eutylenchus excretorius*	*Cephalenchus hexalineatus*
n	20	9
L	820 ± 20.9 (791-858) 2.55	458 ± 43.7 (412-499)
a	39.7 ± 1.4 (37.5-41.9) 3.64	22.2 ± 2.4 (20.6-24.9)
b	6.3 ± 0.3 (5.8-6.9) 5.37	4.7 ± 0.3 (4.4-4.9)
c	7.4 ± 0.3 (6.8-7.8) 4.2	4.8 ± 0.4 (4.4-5.3)
c′	9.0 ± 0.5 (8.3-9.8) 5.79	7.4 ± 0.5 (6.9-7.8)
V	73.4 ± 0.8 (72-74) 1.15	68.0 ± 1.0 (67-69)
G_1	36 ± 3.8 (30-42) 10.49	37 ± 2.2 (35-39)
Stylet length	21.0 ± 0.6 (20.0-22.0) 2.75	15.5 ± 0.5 (15.0-16.0)
O	9.9 ± 0.9 (9.3-11.4) 9.85	10.4 ± 2.1 (8.4-12.5)
Anterior end to excretory pore (EP)	90 ± 4.2 (83-98) 4.69	77 ± 3.0 (74-80)
EP / L × 100%	11.1 ± 0.6 (10.2-11.7) 5.05	16.9 ± 1.9 (14.8-18.7)
EP / pharynx length × 100%	69.8 ± 2.3 (66.2-74.0) 3.37	79.5 ± 5.4 (73.2-83.3)
Anterior end to nerve ring	74.8 ± 5.8 (67-86) 7.69	56.7 ± 1.2 (56-58)
MB	41.3 ± 1.6 (39.0-44.0) 3.95	40.0 ± 1.0 (39.0-41.0)
Pharynx length	131 ± 7.3 (119-144) 5.59	97 ± 3.6 (94-101)
Post-vulval uterine sac	25 ± 1.1 (23-27) 4.53	9 ± 0.6 (9-10)
Vulva-anus distance	104 ± 5.9 (98-113) 5.64	59 ± 1.0 (58-60)
Tail length	111 ± 3.7 (107-118) 3.33	95 ± 1.5 (93-96)

paratus (2.0-2.5) μm long. Isthmus slender 40 ± 4.7 (33-45) μm long, encircled by nerve ring at mid-point. Excretory pore at mid-isthmus level, mostly two annuli posterior to hemizonid, duct weakly cuticularised. Deirids not seen. Basal bulb elongate-saccate, offset from intestine, 30.4±1.9 (27-33) × 9.6±0.5 (9-10) μm. Cardia rounded, 4-5 μm long. Ovary with single row of oocytes. Spermatheca poorly developed, lacking sperm. Ventral cuticular ridges slightly wider at vulval region. Lateral vulval membranes forming advulval flaps. Post-vulval uterine sac 1.2 ± 0.1 (1.1-1.2) times vulval body diam. Tail slender, *ca* as long as vulva to anus distance, tapering to a fine terminus. Longitudinal ridges ending in first third of tail, remainder of tail finely to minutely transversely annulated.

Remarks

When comparing all the morphometric characters from the Spanish population of *E. excretorius* they agree very well with the original description, the redescription of the species by Brzeski (1996) from Poland and three progenies originating from single females that were collected from the rhizosphere of birch (*Betula pendula* Roth.) in the Czech Republic (Háněl, 2000). Nevertheless, some characters and ratios such as V, L, stylet length, tail length, excretory pore position as a percent of pharynx length, a, and MB showed a lower variability than reported by Brzeski (1996). The reduced spermatheca, as well as the absence of sperm and males in the present population, confirms the parthenogenetic reproduction of this species. Likewise, the coefficients of variation for the majority of the characters and ratios characterising the Spanish population of *E. excretorius* were quite similar to those reported by Brzeski (1996) for a population from Poland. The low intraspecific variability of these characters indicates that they may be of primary value for species identification in the genus. Our LM and SEM studies confirm that this species has different cuticular structures near the vulva, a fact which clearly justifies the separation from *E. africanus* and *E. setiferus*.

The present record of *E. excretorius* is the first from Spain and southern Europe and the fifth in Europe after those from Germany (Sievert & Sturhan, 1994), Poland, the Czech Republic and Russia (Brzeski, 1996; Háněl, 2000). The current geographical distribution of *E. excretorius* indicates that it may be mostly associated with cooler regions of the northern hemisphere. Conversely, except for a record from India (Husain & Khan, 1968), *E. africanus* appears to be mostly associated with warmer regions of the southern hemisphere.

Cephalenchus hexalineatus (Geraert, 1962) Golden, 1971
(Fig. 3)

Measurements

See Table 1.

Remarks

The genus *Cephalenchus* is characterised by the generally separated lip region, long, thin stylet and lateral fields with six, rarely four, incisures at mid-body, reducing to four in post-vulval region. It comprises *ca* 20 nominal species (Siddiqi, 2000). The genus is distributed worldwide with the most widely distributed species being *C. megacephalus* (Goodey, 1962) Andrássy, 1984 (Europe, Asia, Africa, Australia) and *C. hexalineatus* (Africa, North America, Australia) (Andrássy, 1984). *Cephalenchus* spp. feed on root epidermal cells of herbaceous and woody plants but, since they do not cause severe damage, are not considered as important plant parasites, except for some examples in conifers (Gowen, 1970; Stoen *et al*., 1988).

The specimens (only females and juveniles were found) of the present populations are characterised by a short stylet with rounded knobs, basal pharyngeal bulb elongate, asymmetric, with slightly lobed posterior margin and about as long as isthmus, vulva with small lateral membranes, short post-vulval uterine sac (shorter than corresponding body diam.), tail filiform with finely rounded tip and 1.5-1.6 times vulva-anus distance and 6.8-7.8 times anal diam. (Fig. 3). Morphology and morphometry of the studied specimens agree very well with previous descriptions of *C. hexalineatus* (Geraert, 1962, 1968; Goodey, 1962; Andrássy, 1984). Nevertheless, small differences in body length and derived ratios (a, b, c), were detected which confirm specific variability as indicated by Raski and Geraert (1986).

Molecular characterisation of *E. excretorius* and phylogenetic position within Tylenchida

The alignment lengths for D2-D3, 18S and hsp90 sequences were 726 bp, 1781 bp and 246 bp, respectively. The sequence of the D2-D3 expansion segments of 28S rRNA from *E. excretorius* from Spain was identical to one from a population from Germany. Phylogenetic trees reconstructed by the BI method for the two rRNA genes (18S rRNA and D2-D3 expansion regions of 28S rRNA gene) are presented in Figure 4. The phylogenetic trees obtained were generally congruent with those given by Bert *et al*. (2008) and by Subbotin *et al*. (2006) for 18S rRNA and D2-D3 28S rRNA phylogenies, respectively. *Eutylenchus excretorius* clustered with moderate support (PP = 90) with *C. hexalineatus* in both rRNA trees. The position of *E. excretorius* on majority consensus BI phylogenetic tree reconstructed using hsp90 gene sequences was not well resolved (Fig. 5). In some BI trees obtained after exclusion of the third nucleotide positions, *E. excretorius* formed a clade with *C. hexalineatus* (PP = 5). Thus, the position of *E. excretorius* inferred from hsp90 gene phylogeny does not conflict with phylogenies reconstructed using rRNA genes. *Macrotrophurus arbusticola*, another representative of the Tyloderinae *sensu* Maggenti *et al.* (1987) clustered with high PP in the 18S tree with nematodes of the subfamily Telotylenchinae Siddiqi, 1960 *sensu* Siddiqi, 2000.

The results of the present phylogenetic analyses support Maggenti *et al*. (1987) and Geraert and Raski (1987) in grouping *Eutylenchus* with *Cephalenchus* based on several congruent morphological characters, *viz*., *i*) labial plate with four sectors and with either four cephalic papillae (*Cephalenchus*) or four setae (*Eutylenchus*) and oral disc with six papillae and amphidial slits longitudinally orientated; *ii*) stylet size (longer than usual in other genera in Tylenchidae) and morphology (anterior part about equal to posterior part) and stylet knobs rounded and well developed; *iii*) pharynx (median bulb well developed and anteriorly situated and glands elongated, symmetrically arranged) and *iv*) female reproductive system with uterus subdivided into a few cells forming the transition zone with the uterine sac and crustaformeria part with five or six cells in each of the four rows (Geraert & Raski, 1987; Maggenti *et al*., 1987). The present results are also congruent with a previous statement (Subbotin *et al*., 2006) that the subfamily Tylodorinae *sensu* Maggenti *et al.* (1987) is not monophyletic. Thus, molecular approaches support the phylogenetic relationships demonstrated by morphological or biological traits and therefore support the inclusion of *Eutylenchus* and *Cephalenchus* in the same group. However, additional analyses with other genes and taxa are still required to resolve the relationships of *Eutylenchus* with nematodes from the families Tylodorinae and Tylenchidae.

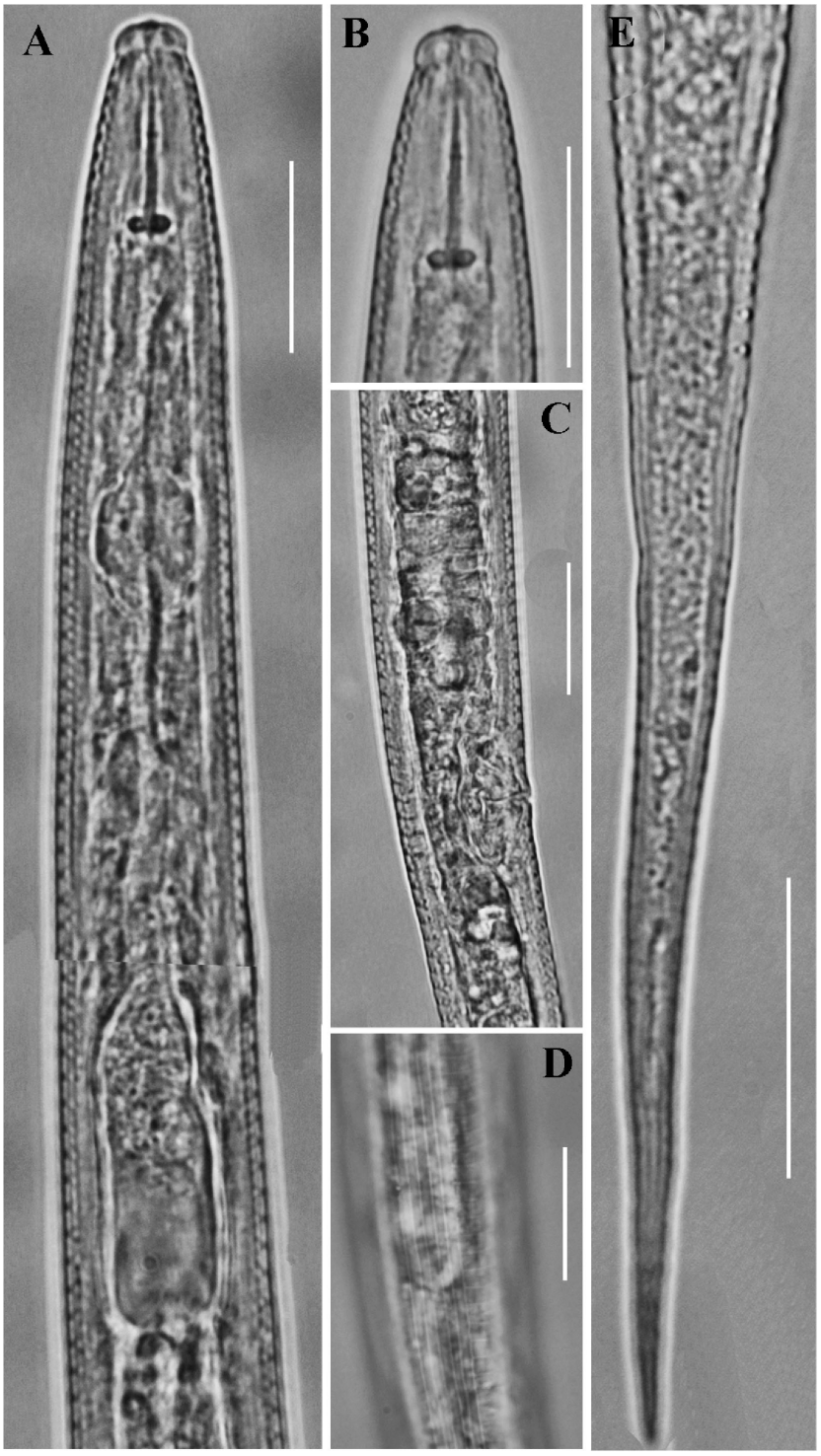

Fig. 3. *Light micrographs of female* Cephalenchus hexalineatus *(Geraert, 1962) Golden, 1971. A: Anterior region; B: Detail of lip region; C: Mid-body region showing vulva and post-vulval uterine sac; D: Mid-body region showing six lateral field incisures; E: Tail region. (Scale bars: A, B = 15 μm; C-E = 20 μm.)*

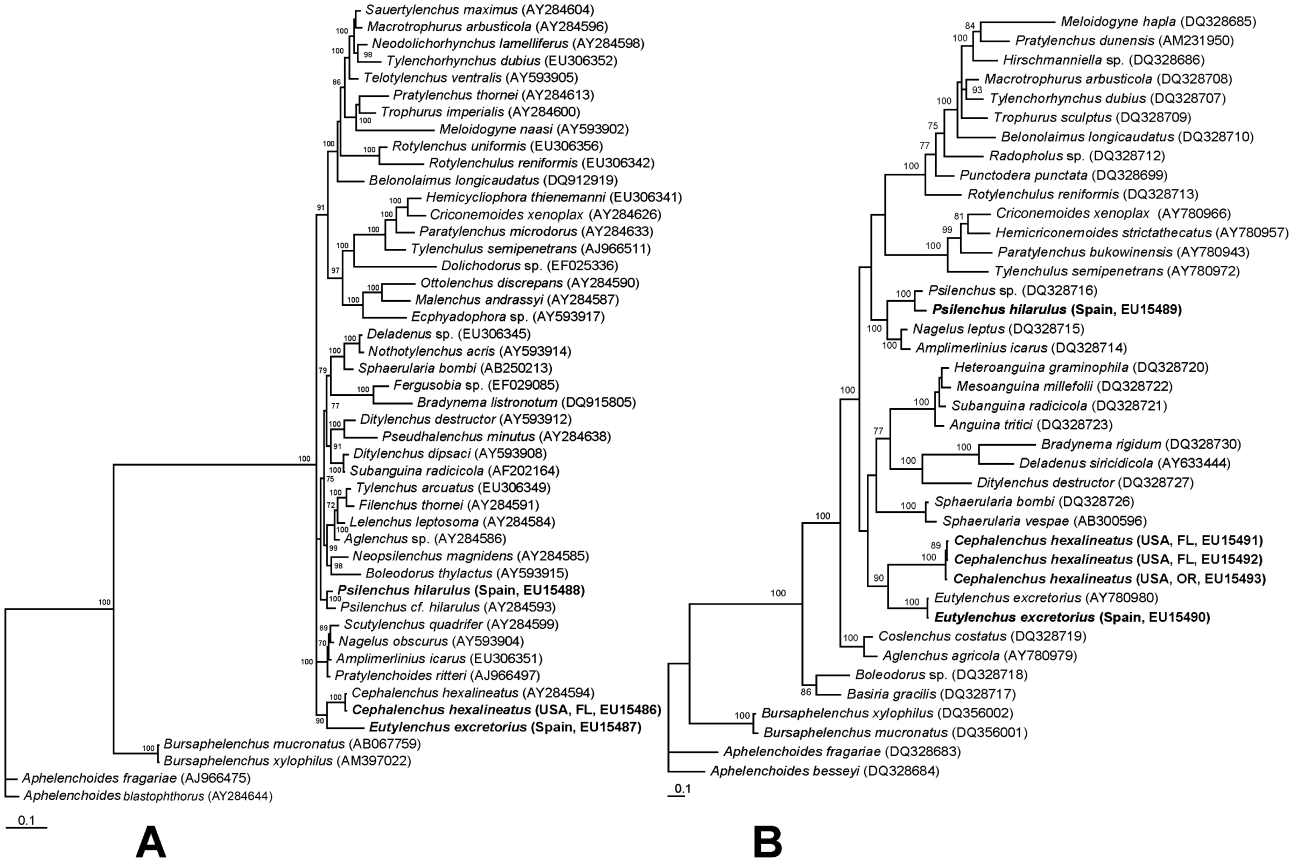

Fig. 4. *Phylogenetic relationships within some Tylenchida species: Bayesian 50% majority rule consensus tree from two runs as inferred from (A) partial 18S rRNA gene and (B) D2-D3 of 28S gene sequence alignments under the GTR + I + G model. Posterior probabilities more than 70% are given for appropriate clades. Newly obtained sequences are indicated by bold letters.*

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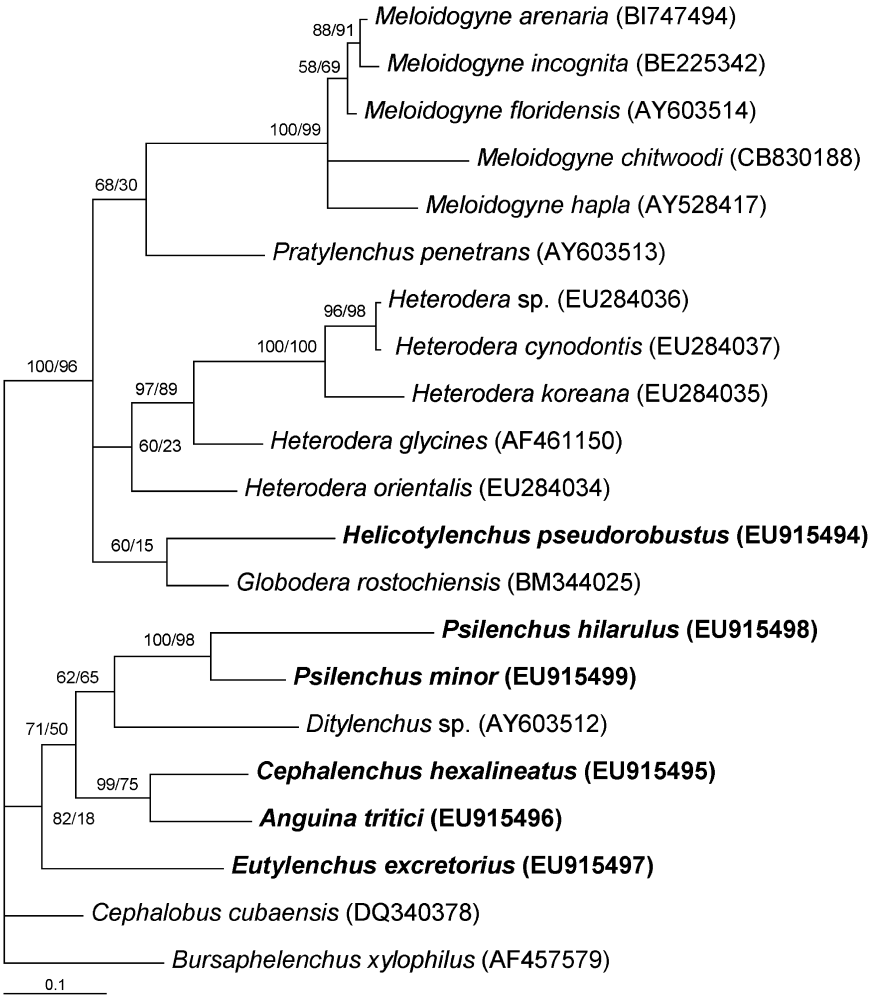

Fig. 5. *Phylogenetic relationships within some Tylenchida species: Bayesian 50% majority rule consensus tree from two runs as inferred from hsp90 gene sequence alignment under the GTR + I + G model. Posterior probabilities for the full dataset/the dataset after exclusion of the third nucleotide positions are given for appropriate clades. Newly obtained sequences are indicated by bold letters.*

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